DOCUMENT-IDENTIFIER: US 20020198372 A1

TITLE: METHODS FOR PURIFYING NUCLEIC ACIDS

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Brief Description of Drawings Paragraph - DRTX (22):

[0042] The preferred method for purifying plasmid DNA at larger scale is to load the lysate directly onto the ion exchange chromatography column after clarification and neutralization. This can be done to avoid additional purification steps, and greatly simplifies the process. In this "direct load" process, after the lysate is clarified by centrifugation, further debris may be removed by, e.g., decanting through a depth filter. The pH and conductivity is then adjusted to the appropriate values, which depend on the anion exchange resin used. A preferred resin is TMAE Fractogel 650M resin (EM Separations Technology, Wakefield, R.I., US Associate of E. Merck, Darmstadt, Germany). With this resin, he pH is adjusted to about 8.5 and the conductivity is adjusted to less than about 50 mS/cm. This can be accomplished by adding about 0.6 volumes of 1M Tris per volume of lysate or by diluting 0.25-0.3 fold with water, then adding Tris base powder to a final concentration of 0.62M.

DOCUMENT-IDENTIFIER: US 20020156037 A1

TITLE: DNA vaccine formulations

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Detail Description Paragraph - DETX (73):

[0126] 4.5 L of frozen E. coli cell slurry was used to make 33.7 L of cell suspension in STET buffer (8% sucrose, 2% Triton, 50 mM Tris buffer, 50 mM EDTA, pH 8.5) with 2500 units/ml of lysozyme. The absorbance of the suspension at 600 nm was about O.D. 30. The suspension was stirred at room temperature for 15 minutes to ensure proper mixing and then was incubated for 45 minutes with continuous stirring at 37.degree. C. Following incubation, mixing was continued at room temperature and the cell suspension was pumped through the heat exchanger at a flowrate of 500 ml/min. The batch temperature was maintained at 100.degree. C. and the inlet and outlet temperatures of the cell suspension were measured to be about 24.degree. C. and between 70-77.degree. C., respectively. The cell lysate exiting the heat exchanger was collected in Beckman centrifuge bottles (500 mls each) and the material was centrifuged immediately in Beckman J-21 centrifuges for 50 minutes at 9000 RPM. Following centrifugation, the supernatant was found to contain 4-5 times more plasmid product than in the case without lysozyme incubation. The supernatant product of the centrifugation was immediately diafiltered against 3 volumes of TE buffer (25 mM Tris-EDTA at pH 8.0) and then incubated with 20.times.10.sup.5 units of E. coli RNase for 2-4 hours at room temperature. After completion of the incubation, the product solution was then diafiltered an additional 6 volumes with TE buffer using a 100 kD MWCO membrane and then filtered through a 0.45 micron filter to remove residual debris. The filtered lysate was diluted to 0.7 M NaCl with a 20 mM Bis/Tris Propane-NaCl buffer at pH 7.5, which prepares the diluted filtrate for loading onto the anion exchange column. The anion exchange column (3.6 L of POROS PI/M) was previously equilibrated

with 20
mM Bis/Tris Propane and 0.7M NaCl. The filtered lysate was loaded to column
capacity. In this case 5 grams of supercoiled plasmid was loaded onto the
anion exchange column. After loading, the column was washed with 2-4 column
volumes of 20 mM Bis/Tris Propane and 0.7 M NaCI. A 10 column volume gradient
from 0.7 M NaCl to 2.0 M NaCl in 20 mM Bis/Tris Propane was performed to clear
most of the E. coli protein. RNA and some endotoxin. The supercoiled plasmid
fraction eluted between 1.4 M and 2.0 M NaCl. The supercoiled fraction from
the anion exchange column, which contained 4 grams of supercoiled plasmid was
then diluted 2-3 times with pyrogen free water, adjusted to 1.2% IPA and pH
adjusted to 8.5 with 1 N NaOH. The diluted anion exchange supercoiled fraction
was then loaded onto a 7 L reversed phase column (POROS R2/M) which had been
previously equilibrated with 100 mM Ammonium Bicarbonate containing 1.2% IPA.
In this case, 3.2 grams of supercoiled plasmid were loaded onto the reversed
phase column and then the column was washed with 6-10 column volumes of 1.2%
IPA in 100 mM Ammonium Bicarbonate. This extensive wash was performed to clear
impurities. Next, a gradient of 1.2% IPA to 11.2% IPA in 5 column volumes was
performed. The supercoiled plasmid fraction elutes at about 4% IPA. The
supercoiled product fraction from the reversed phase column was then
concentrated and diafiltered into normal saline using a 30 kD MWCO membrane.
The final product bulk was filtered through a 0.22 micron filter. The overall
product yield of the process was more than 50% of the supercoiled plasmid in
the clarified cell lysate as indicated by the anion exchange HPLC assay.

DOCUMENT-IDENTIFIER: US 20020032324 A1

TITLE: Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy

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Detail Description Paragraph - DETX (15):

[0063] A 150 ml HB 101 E. coli culture with pUC 18 plasmid DNA in LB medium is centrifuged at 3000.times. g for 5 min to pelletize the cells. The cell pellet is resuspended in 20 ml of 50 ml Tris/HCl, 10 mM EDTA, pH 8.0, 100 .mu.g/ml RNase A. Twenty milliliters of 0.2 M NaOH, 1% SDS are added to the cell suspension for cell lysis, cautiously mixed and kept standing at room temperature for 5 minutes. Then, 20 ml of 3 M potassium acetate, 2 M acetic acid is added for neutralisation, mixed, and incubated on ice for 15 minutes, and the cell lysate is sucked through the filter device according to the invention at a pressure difference of 20 mbar to 800 mbar. Alternatively, the sample may be pressed through the filter layers with a piston or by increased pressure. After the filtration, the filtration device is removed, and the filter cake with the cell fragments, denatured proteins and precipitated SDS is discarded. The filtrated lysate is mixed with [fraction (1/10)] of its volume of Endotoxin Removal Buffer (750 mM NaCl; 10% Triton X 114; 40 mM MOPS, pH 7.0) and incubated on ice for 30 min. The filtrate is completely sucked or pressed through the anion exchange column to achieve adsorption of the DNA. The extraction column is subsequently washed twice with 100 ml of 1 M NaCl, 15% ethanol, 50 mM MOPS, pH 7.0, to remove RNA and proteins. The DNA is eluted with 100 ml of 1.6 M NaCl, 15% ethanol, 50 mM MOPS, pH 7.0. The eluted DNA is precipitated with alcohol for desalting and concentrating, and the alcoholic pellet is pelletized by centrifugation.

US-PAT-NO: 6551556

DOCUMENT-IDENTIFIER: US 6551556 B1

TITLE: Automatic DNA purification apparatus

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Detailed Description Text - DETX (21):
Through injection needles (26), various biological liquid samples which contain DNA are transferred to the first filter (trapping filter) plate. The second filter plate (trapping plate) is disposed in lower part of inside of vacuum container (42). The first filter plate (binding plate) is disposed over the second filter plate. Then, vacuum block (41) is operated to filter in vacuo the liquids contained in the first filter plate. Cell debris, proteins, genomic DNA and the like are retained within the first filter plate and liquids which contain plasmid DNA are collected into the second filter plate.

Detailed Description Text - DETX (23):
Then, the recovering filter plate is disposed in lower part of inside of vacuum container (42). The second filter plate which contains plasmid DNA thus dried, is disposed over said recovering filter plate. Through injection needles (26), elution buffer such as Tris-HCl solution are injected into each well of the second filter plate in order to desorb plasmid DNA from binding filter. Then, vacuum block (41) is operated to collect purified plasmid DNA into the recovering plate.

US-PAT-NO: 6387695

DOCUMENT-IDENTIFIER: US 6387695 B1

TITLE: DNA pharmaceutical formulations comprising citrate or triethanolamine and combinations thereof

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Detailed Description Text - DETX (32):
Multi-Gram Scale Purification of Plasmid DNA--4.5 L of frozen E. coli cell slurry was used to make 33.7 L of cell suspension in STET buffer (8% sucrose, 2% Triton, 50 mM Tris buffer, 50 mM EDTA, pH 8.5) with 2500 units/ml of lysozyme. The absorbance of the suspension at 600 nm was about O.D. 30. The suspension was stirred at room temperature for 15 minutes to ensure proper mixing and then was incubated for 45 minutes with continuous stirring at 37.degree. C. Following incubation, mixing was continued at room temperature and the cell suspension was pumped through the heat exchanger at a flow rate of 500 ml/min. The batch temperature was maintained at 100.degree. C. and the inlet and outlet temperatures of the cell suspension were measured to be about 24.degree. C. and between 70-77.degree. C., respectively. The cell lysate exiting the heat exchanger was collected in Beckman centrifuge bottles (500 mls each) and the material was centrifuged immediately in Beckman J-21 centrifuges for 50 minutes at 9000 RPM. Following centrifugation, the supernatant was found to contain 4-5 times more plasmid product than in the case without lysozyme incubation. The supernatant product of the centrifugation was immediately diafiltered against 3 volumes of TE 35 buffer (25 mM Tris-EDTA at pH 8.0) and then incubated with 20.times.10.sup.5 units of E. coli RNase for 2-4 hours at room temperature. After completion of the incubation, the product solution was then diafiltered an additional 6 volumes with TE buffer using a 100 kD MWCO membrane and then filtered through a 0.45 micron filter to remove residual debris. The filtered lysate was diluted to 0.7 M NaCl with a 20 mM

Bis/Tris Propane-NaCl buffer at pH 7.5, which prepares the diluted filtrate for loading onto the anion exchange column. The anion exchange column (3.6 L of POROS PI/M) was previously equilibrated with 20 mM Bis/Tris Propane and 0.7M NaCl. The filtered lysate was loaded to column capacity. In this case 5 grams of supercoiled plasmid was loaded onto the anion exchange column. After loading, the column was washed with 2-4 column volumes of 20 mM Bis/Tris Propane and 0.7 M NaCl. A 10 column volume gradient from 0.7 M NaCl to 2.0 M NaCl in 20 mM Bis/Tris Propane was performed to clear most of the E. coli protein, RNA and some endotoxin. The supercoiled plasmid fraction eluted between 1.4 M and 2.0 M NaCl. The supercoiled fraction from the anion exchange column, which contained 4 grams of supercoiled plasmid was then diluted 2-3 times with pyrogen free water, adjusted to 1.2% IPA and pH adjusted to 8.5 with 1 N NaOH. The diluted anion exchange supercoiled fraction was then loaded onto a 7 L reversed phase column (POROS R2/M) which had been previously equilibrated with 100 mM Ammonium Bicarbonate containing 1.2% IPA. In this case, 3.2 grams of supercoiled plasmid were loaded onto the reversed phase column and then the column was washed with 6-10 column volumes of 1.2% IPA in 100 mM Ammonium Bicarbonate. This extensive wash was performed to clear impurities. Next, a gradient of 1.2% IPA to 11.2% IPA in 5 column volumes was performed. The supercoiled plasmid fraction elutes at about 4% IPA. The supercoiled product fraction from the reversed phase column was then concentrated and diafiltered into normal saline using a 30 kD MWCO membrane. The final product bulk was filtered through a 0.22 micron filter. The overall product yield of the process was more than 50% of the supercoiled plasmid in the clarified cell lysate as indicated by the anion exchange HPLC assay.

US-PAT-NO: 6297371

DOCUMENT-IDENTIFIER: US 6297371 B1

TITLE: Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy

---------- KWIC ---------

Detailed Description Text - DETX (15):
A 150 ml HB 101 E. coli culture with pUC 18 plasmid DNA in LB medium is centrifuged at 3000.times. g for 5 min to pelletize the cells. The cell pellet is resuspended in 20 ml of 50 ml Tris/HCl, 10 MM EDTA, pH 8.0, 100 .mu.g/ml RNase A. Twenty milliliters of 0.2 M NaOH, 1% SDS are added to the cell suspension for cell lysis, cautiously mixed and kept standing at room temperature for 5 minutes. Then, 20 ml of 3 M potassium acetate, 2 M acetic acid is added for neutralisation, mixed, and incubated on ice for 15 minutes, and the cell lysate is sucked through the filter device according to the invention at a pressure difference of 2000 Pa to 80,000 Pa (20 mbar to 800 mbar) . Alternatively, the sample may pbas pressed through the filter layers with a piston or by increased pressure. After the filtration, the filtration device is removed, and the filter cake with the cell fragments, denatured proteins and precipitated SOS is discarded. The filtrated lysate is mixed with 1/10 of its volume of Endotoxin Removal suffer (750 mM NaCl; 10 Triton.RTM. X 114; 40 mM MOPS, pH 7.0) and incubated on ice for 30 min. The filtrate is completely. sucked or pressed through the anion exchange column to achieve adsorption of the DNA. The extraction column is subsequently washed twice with 100 ml of 1 M Nacl, 15% ethanol, 50 mM MOPS, pH 7.0, to remove RNA and proteins. The DNA is eluted with 100 ml of 1.6 M NaCl , 15% ethanol, 50 mM MOPS, pH 7.0. The eluted DNA is precipitated with alcohol for desalting and concentrating, and the alcoholic pellet is pelletized by centrifugation.